The wells were blocked with 200 μL 3% bovine serum albumin (BSA) in PBS for at least 2 hours at room temperature. The wells were then washed 3 times with TBST, incubating each step for 5 min. Culture supernatant dilutions were prepared in 3% BSA in PBS, and 50 microliters of the dilutions were added to each well and incubated for 1 hour. The wells were washed 3 times with TBST for 5 min each. The secondary rat anti-mouse antibody horseradish peroxidase (HRP) conjugate (clone 23G3, Southern Biotech, Birmingham, Ala.) was prepared in 3% BSA in PBS at 1:1000 dilution, added to the wells and incubated for 1 h at room temperature. The wells were washed 3 times with TBST for 5 min each, and 100 microliters of fresh substrate (o-phenylenediamine dihydrochloride, Pierce, Rockford, Ill.) in buffer solution is added to each well. The absorbance at 450 nm was read every 10 seconds for 10 min using the kinetic measurement mode of a plate reader. The measured signal was plotted as a function of time, and the initial slope was determined which provides a measure of the relative antibody concentration. The control signal obtained from wells with no protein was subtracted from the measured values.

[0204] Several distinct components were used for the all-microfluidic approach to single cell experiments: encapsulation, incubation, and manipulation devices, as indicated by the boxes in FIG. **6**.

[0205] To illustrate the utility of this modular approach to drop based cell handling, a line of hybridoma cells which secrete anti-ovalbumin IgE antibodies was used. These hybridomas are suspension cells simplifying their handling in drops.

[0206] The cell encapsulation device used a flow focusing geometry to produce drops, as shown schematically on the left of FIG. **6***a*. Additional inlets can be incorporated on chip to mix reagents with the cells just before they are encapsulated, as shown schematically on the right of FIG. **6***a*. Three inlet channels, coming from the left, convert to form a nozzle as shown in the optical micrographs in FIGS. **6***b* and **6***c*. In both cases, the center stream contains the cell suspension while the side streams contain the oil phase. The drop volume can easily be varied between about 0.5 pL and about 1.8 nL, corresponding to spherical drops of diameter 10 microns to 150 microns. This was accomplished by matching the size of the nozzle orifice to the drop diameter and operating the device in the dripping regime. Fine tuning of the drop size for a given nozzle can be accomplished by varying the inner, aqueous flow rate or the overall flow rate; this also leads to variation in the drop production frequency. The modular nature of the device enables the nozzle dimension, and hence the drop size, to be readily changed without affecting any other components.

[0207] Individual syringe pumps were used to control the flow of the oil and the cell suspension. In this set of embodiments, the focus is on suspension cells; however, adherent cells can also be studied by first growing the cells on small beads and then encapsulating the beads. To prevent settling of the cells and maintain the desired density, the suspension was stirred constantly. Typically a 5 mL syringe containing 1 mL of cell suspension was used, ensuring that the depth of the volume was comparable to its height, thus enabling it to be easily mixed using a small magnetic stir bar. A convenient method of stirring the sample, while preventing clogging of the syringe, was to maintain it at a 45° upward angle and to place a stir plate on top of it. Using this scheme the encapsulation efficiency was typically approximately 70%. Account for this factor, one can reliably and reproducibly obtain the desired cell distribution in the drops. Single-cell studies require that most or all drops contain at most one cell, so that the majority of drops contain no cell at all since the encapsulation process follows Poisson statistics. Production of drops encapsulating individual cells is shown in FIG. **7***a*, where black arrows highlight the cell-bearing drops. The Poisson distribution for cells is given by:

$$f(\lambda, n) = \frac{\lambda^n e^{-\lambda}}{n!}$$

where n is the number of cells in the drop, and lambda is the average number of cells per drop; lambda can be adjusted by controlling the cell density. The distributions of cells in drops for lambda=0.1, 0.3, and 0.5 were demonstrated; these are typical values of interest for single cell experiments as they ensure that very few drops contain multiple cells. In each case, the results were in good agreement with those calculated from Poisson statistics for the values of lambda used, as shown in FIG. **7***b*. By using lambda=0.3, cells were observed in roughly 22% of the drops, and fewer than 4% of the drops included two or more cells. Although the number of single-cell-bearing drops was rather low, the effect was not severe in this set of embodiments, given the high production and screening rate that could be achieved with microfluidic devices.

[0208] The incubation device included a long serpentine channel with a volume of 144 microliters, enabling it to hold a large quantity of drops, as shown schematically in the top of FIG. **6***d*. Cell-bearing drops produced in the encapsulation device could be redirected into the incubation device by means of external tubing. Inside the device the flow rate of the carrier oil was faster than that of the drops, thereby concentrating the emulsion. Interestingly, because of their buoyancy the drops collected at the top of the channel where they formed a well-packed single layer, as shown in FIGS. **6***e* and *f*. Despite the high packing of the drops, the surfactant ensured stability, and virtually no uncontrolled coalescence was observed.

[0209] The incubation device could be detached from the encapsulation device and placed in a cell incubator or other storage container to maintain the desired temperature and gas atmosphere. By carefully maintaining the channels filled with oil, any deleterious effects of air in the channels could be avoided. The permeability of both the PDMS and the fluorocarbon carrier oil to gas enabled sufficient exchange to keep the cells at the level set by the environment; this was facilitated by their monolayer packing. The water saturated atmosphere prevented evaporation of water from the drops ensuring they retained the desired size and concentration. Independent studies over long periods of time confirmed that the drop diameter shrank by less than 3.5% after 72 hours; thus, for the much shorter incubation times used in these experiments, it was determined that the shrinkage was negligible.

[0210] To ascertain cell viability, the emulsion was broken after incubation, the cells were recovered, and live-dead assays were performed. After incubation for a period of 6 hours, it was determined that the cells had a survival rate of approximately 85%; by comparison, an identical survival rate was found for cells incubated on culture dishes as shown in FIG. **8***a*. Maintaining the cells in drops and on chip for all